Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

tembotrione

EC number: N/A

CAS number: 335104-84-2

CLH-O-0000002527-72-03/F

Adopted

4 June 2013

4 June 2013

CLH-O-0000002524-78-03/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: tembotrione

EC number: N/A

CAS number: 335104-84-2

The proposal was submitted by **Austria** and received by the RAC on **14/05/2012.**

In this opinion, all classifications are given firstly in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS) and secondly, according to the notation of 67/548/EEC, the Dangerous Substances Directive (DSD).

PROCESS FOR ADOPTION OF THE OPINION

Austria has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **14/05/2012**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **28/06/2012**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Bert-Ove Lund**

Co-rapporteur, appointed by RAC: **José Luis Tadeo**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation.

The RAC opinion on the proposed harmonised classification and labelling was reached on **4 June 2013** and the comments received are compiled in Annex 2.

The RAC Opinion was adopted by **consensus**.

OPINION OF THE RAC

The RAC adopted the opinion that tembotrione should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)	
Current Annex VI entry	-	-	-	-	-	-	-	-	-	-
Dossier submit-ters proposal	606-149-00-3	tembotrione (ISO); 2-{2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl}cyclohexane-1,3-dione	-	335104-84-2	STOT RE 2 Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 1	H373 H317 H400 H410	GHS09 Wng	H373 H317 H410		M=100 M=10
RAC opinion	606-149-00-3	tembotrione (ISO); 2-{2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl}cyclohexane-1,3-dione	-	335104-84-2	Repr. 2 STOT RE 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H361d H373 (eyes, kidneys, liver) H317 H400 H410	GHS08 GHS07 GHS09 Wng	H361d H373 (eyes, kidneys, liver) H317 H410		M=100 M=10
Resulting Annex VI entry if agreed by COM	606-149-00-3	tembotrione (ISO); 2-{2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl}cyclohexane-1,3-dione	-	335104-84-2	Repr. 2 STOT RE 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H361d H373 (eyes, kidneys, liver) H317 H400 H410	GHS08 GHS07 GHS09 Wng	H361d H373 (eyes, kidneys, liver) H317 H410		M=100 M=10

Classification and labelling in accordance with the DSD

	Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits
Current Annex VI entry	-	-	-	-	-	-	-
Dossier submit-ters proposal	606-149-00-3	tembotrione (ISO); 2-{2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl}cyclohexane-1,3-dione	-	335104-84-2	Xi; R43 Xn; R48/22 N; R50-53	Xi; Xn; N R: 42-48/22-50/53 S:	N; R50-53: C ≥ 2,5% N; R51-53: 0.25% ≤ C < 2,5% R52-53: 0.025% ≤ C < 0,25%
RAC opinion	606-149-00-3	tembotrione (ISO); 2-{2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl}cyclohexane-1,3-dione	-	335104-84-2	Repr. Cat. 3; R63 Xn; R48/22 R43 N; R50-53	Xn; N R: 43-48/22-50/53-63 S: (2-)36/37-46-60-61	N; R50-53: C ≥ 0,25 % N; R51-53: 0,025 % ≤ C < 0,25 % R52-53: 0,0025 % ≤ C < 0,025 %
Resulting Annex VI entry if agreed by COM	606-149-00-3	tembotrione (ISO); 2-{2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl}cyclohexane-1,3-dione	-	335104-84-2	Repr. Cat. 3; R63 Xn; R48/22 R43 N; R50-53	Xn; N R: 43-48/22-50/53-63 S: (2-)36/37-46-60-61	N; R50-53: C ≥ 0,25 % N; R51-53: 0,025 % ≤ C < 0,25 % R52-53: 0,0025 % ≤ C < 0,025 %

SCIENTIFIC GROUNDS FOR THE OPINION

RAC general comment

The RAC has evaluated only those hazard classes: a) for which a classification was proposed by the dossier submitter (DS), b) for which comments were received during the public consultation and data was made available or, c) those which were specifically requested by the RAC. Any other hazard classes related to this substance should be considered as **'not evaluated'** and their exclusion should not be taken to mean 'not classified'.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

The CLH report refers to one Magnusson and Kligman Maximisation test on skin sensitisation in Guinea-pigs (Coleman, 2003, see BD) performed according to OECD guideline 406. Because of technical problems (bandage lost from 2 animals), eight animals were used for the final challenge. All eight responded positively to the challenge with 50 or 100% tembotrione (in 1:1 Alembicol D and complete Freund's adjuvant), whereas none of the five controls reacted to the vehicle. As more than 30% of the animals reacted to a concentration of 50 or 100% tembotrione, the dossier submitter concluded that tembotrione is a skin sensitiser and proposed classification with Skin Sens. 1B; H317 and R43.

Comments received during public consultation

The CLH report variously proposed both for Skin Sens.1 (without specifying 1A or 1B) and Skin Sens. 1B. Four comments were received, all from MS, and they agreed with classifying tembotrione as a skin sensitiser, albeit two agreed with category 1 and two with category 1B.

Assessment and comparison with the classification criteria

The RAC considered that the above study showed a sensitisation potential of tembotrione. After 24 hours, the average erythema score was 1 (discrete or patchy). The effects became more severe with time, with grade 2 (moderate and confluent) erythema in all animals 48 h after the challenge with 50% tembotrione (and an average score of 1.6 with 100% tembotrione). As the intra-dermal induction dose was >1% (i.e. 2.5%), the data support classification in sub-category 1B. However, with such a high level of responders (100%) after intradermal induction with 2.5%, there is a possibility that at a slightly lower intradermal induction concentration of 1% there will still be a considerable level of responders, potentially over 60% (which would support classification in category 1A). Lower intradermal induction concentrations than 2.5% have however not been tested, so in principle the data are insufficient for sub-categorisation.

The RAC thus concluded that classification of tembotrione with Skin Sens. 1; H317 (R43 according to DSD) was warranted.

RAC evaluation of specific target organ toxicity (CLP) – repeated exposure (STOT RE) and repeated dose toxicity (DSD)

Summary of the Dossier submitter's proposal

The DS based the evaluation of STOT RE on 9 studies, two in mice, four in rats, two in dogs, and one in rabbits.

In rats, specific ocular lesions were observed at very low doses (LOAEL 0.8-1.0 mg/kg/day in a 2 year oral study). However, mechanistic studies have shown this effect to be caused by a build-up of tyrosine (tyrosinaemia) following inhibition of the enzyme 4-hydroxy-phenylpyruvate dioxygenase (HPPD) by tembotrione. Thus, the tyrosine concentration increases *in vivo* in rats at low exposure to tembotrione. *In vitro* studies have shown tyrosine to build up in rat hepatocytes exposed to tembotrione whereas human (and mouse) hepatocytes can still metabolise tyrosine via alternative pathways. No ocular lesions are seen in mice. Experimental studies in rats have also shown that tyrosine exposure produces the same ocular lesions as tembotrione. Overall, the dossier submitter concluded that the tyrosinaemia-mechanism of action for tyrosinaemia seen in

rats is not relevant for humans, and that no classification for tembotrione based on the ocular lesions is warranted. Other organs, such as the liver, pancreas, and thyroid are affected at higher exposure levels in these studies, but these effects are also suggested by the DS to be related to the tyrosinaemia.

There is only one study in rabbits included in the CLH report, a developmental toxicity study with tembotrione administered by gavage during gestation days 6-28. At a dose of 100 mg/kg/day, 5 out of 25 dams died during gestation days 15-22, indicating severe toxicity at this dose. Although tyrosinaemia also occurs in rabbits, this mechanism was not thought to be involved in the deaths of the dams. Considering the short treatment period in a developmental toxicity study (23 days), the DS compared the effect level with the guidance values for 28-days studies, which state that effects seen below 300 mg/kg/day but above 30 mg/kg/day warrant classification in STOT RE category 2. The dossier submitter therefore proposed classification with STOT RE 2; H373 (May cause damage to organs through prolonged or repeated exposure (if swallowed)) and Xn; R48/22 according to DSD.

Comments received during public consultation

Four comments were received, all from Member States. Three agreed with the proposal. One MS questioned the proposed classification, suggesting that further consideration should be given to the other repeated dose toxicity studies not fulfilling the classification criteria.

Assessment and comparison with the classification criteria

The CLH report describes in total 16 studies with repeated dose exposure, when including studies on carcinogenicity, neurotoxicity and reproductive toxicity. RAC has evaluated all these studies with respect to relevance for STOT RE and repeated dose toxicity. In the repeated dose toxicity studies, the triketone herbicide tembotrione has caused effects on the eyes in rats and dogs, liver (all species), pancreas (rats), the haematological system (dogs and mice), peripheral nerves (rats and dogs), and the kidney (rats).

The ocular toxicity occurred at doses relevant for classification (potentially STOT RE 1). However, mechanistic studies have been provided to suggest that the mode of action for the eye toxicity may not be relevant for humans. This mode of action builds on the observation that tembotrione is a specific inhibitor of 4-hydroxy-phenylpyruvate dioxygenase, leading to an accumulation of tyrosine, which subsequently causes eye toxicity unless it is catabolised by other metabolic pathways. The CLH report does not provide data showing the direct inhibitory effect of tembotrione on HPPD (metabolising tyrosine) from different species, but refers to an in vitro study showing "more" degradation of tyrosine in human hepatocytes than in rat hepatocytes. Increased levels of tyrosine in rats after exposure to tembotrione are also documented.

The RAC has reviewed the literature (see in depth analyses of repeated dose toxicity by RAC in the BD), and is of the opinion that tyrosinaemia is a relevant mode of action (MoA) in humans. The triketone analogue NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione) is used as a pharmaceutical drug to inhibit HPPD, and the potency of tembotrione in humans might not be that much lower than the potency of NTBC. As NTBC has been shown to greatly increase tyrosine concentrations in healthy adult volunteers treated with a single dose of 1 mg/kg/day NTBC (Lock et al, 2001), and to cause eye problems in some children treated with 1 mg/kg/day NTBC against tyrosinaemia type 1, tembotrione can be expected to have an intrinsic possibility to also cause similar problems in humans. Concerning human sensitivity in relation to the animal data, this might be intermediate to that of the very sensitive rat and the non-sensitive mouse. The RAC therefore considers the findings in the rat studies relevant to humans, but with some reservation because of the expected lower sensitivity of humans than of rats.

In rats, tembotrione-induced tyrosinaemia affected the eyes, the pancreas and the liver, with the eye as the primary target organ (NOAEL 0.04-0.1 mg/kg/day). Corneal opacities, neovascularisation and oedema of the cornea, snow flake-like corneal opacities, and keratitis were observed at doses of > about 1 mg/kg/day in a 2 year study. Some of the effects were reversible whereas others (e.g., neovascularisation) appeared irreversible. Hepatic effects were generally rather mild, but fibrosis was noted at doses of > 1 mg/kg/day. The acinar atrophy/fibrosis of the pancreas (no further data given) observed at doses of $\geq$8 mg/kg/day in the

rat 2 year study (and also observed in 28 days dermal rat study), could potentially warrant classification. The guidance values for STOT RE 2 based on a 2-year study are between 1.2 and 12.5 mg/kg/day, and the ocular effects are sufficiently severe and relevant for humans to qualify for a STOT RE classification. As to the category, an effect level of about 1 mg/kg/day is obviously a borderline case, but considering that rats are likely to be more sensitive than humans, RAC considers classification in STOT RE 2 appropriate.

The haematological effects only occur in dogs at doses above the guidance values for classification. Haematological effects are also observed in the 80 weeks mouse study, at doses $\geq$ 4 mg/kg/day, potentially warranting classification. However, no information on the magnitude of these effects is given in the CLH report, and RAC can therefore not assess whether classification for haematological effects is needed.

Neurotoxicity was observed in the 90 days dog study, as determined by clinical signs and histopathological investigation of nerves, but only at and above 120 mg/kg/day, which is above the guidance values for classification. Histopathological lesions in the sciatic nerves were observed at 134 mg/kg/day in the 2 year rat study. Some clinical signs of neurotoxicity were also observed in an acute neurotoxicity study in rats at $\geq$500 mg/kg/day, but not in a 90 days neurotoxicity study in rats (top dose 160/224 mg/kg/day). A developmental neurotoxicity study in rats, with exposure from gestation day 6 until day 21 was also performed. The study is poorly reported in the CLH report. In addition to a decreased growth rate of the pups (magnitude not given) the only finding reported was a decreased acoustic startle response in the pups at 16 and 118 mg/kg/day. No information on dose-response or magnitude of effects is given. Based on the information available to RAC, no classification for neurotoxicity is warranted.

No kidney effects were reported in the studies described in the repeated dose toxicity section of the CLH report (in rats, mice, rabbit or dogs). However, in male rats of the 2 years study, an increased relative kidney weight (see table B.6.5.1-32 in BD under 'In depth analysis of RAC') and histopathological findings were observed at doses $\geq$ 0.8 mg/kg/day (next higher doses were 8.3 and 31.7 mg/kg/day). The effects were characterised as chronic nephropathy, including tubular cell regeneration, thickened basement membranes, interstitial fibrosis, inflammation, dilated/cystic tubules, protein casts, pigmentation, mineralisation, debris, mesangial proliferation, glomerular sclerosis, and hypertrophy/hyperplasia of tubular epithelium. The combined incidences of moderate to severe (sometimes lethal) nephropathy were 4/60, 11/60, 21/60, 23/60 and 19/60 at 0, 0.4, 0.8, 8.3, and 31.7 mg/kg/day, respectively (see in BD 'In depth analysis of RAC'). The effects are sufficiently adverse to warrant classification, but human relevance of this chronic nephropathy has been questioned because of this effect possibly being a specific effect of old male rats. However, RAC notes that chronic nephropathy was also observed in the females, although tembotrione did not aggravate the symptoms in the females.

Although no effects on kidneys were reported in the 28 and 90 days studies in rats, increased relative kidney weights were noted in P0 and F1 males of the 2-generation study in rats. The weights were dose-dependently and statistically significantly increased, exceeding the historical control data from the lowest dose level (1.1-3.3 mg/kg/day). The incidence of dilated renal pelvis was dose-dependently increased in both male (2/30, 4/29, 15/30, and 16/30) and female F1 animals (1/30, 2/29, 4/30, and 17/30), at 0, 20, 200 and 1500 ppm, respectively with the incidence exceeding the historic control data as from the mid dose (13-31 mg/kg/day).

The CLH report provides two arguments why the kidney effects should not be considered; that they are normal findings in ageing rats, and that they are caused by an α2μ-globulin mechanism that is considered specific to male rats and of no relevance for humans. The increased kidney weights in the 2-generation study, where the males did not get very old, may indicate that this effect also can occur in younger animals. When discussing the 2-generation study, the CLH report claims that 86 and 413 mg/kg/day of tembotrione in the 90 days rat study "provoked an accumulation of hyaline droplets in the kidneys without degenerative changes in the tubules which were considered to represent an accumulation of α2μ-globulin". However, no such data are described in the reporting of the 90 days study in the CLH report.

The RAC notes that the EFSA peer review of tembotrione (EFSA Journal, 2013, see BD) did not support the conclusion that the kidney effects are not relevant for humans, that the US EPA did not disregard the kidney effects (US EPA, 2007, see BD) and that the triketone analogue sulcotrione has recently been classified as STOT RE 2 based on kidney effects starting from a dose of 0.04 mg/kg/day and appearing towards the end of the 2 year rat study. The kidney effects reported for sulcotrione were kidney cysts, kidney enlargement, pelvis dilation, and at higher doses chronic progressive nephrosis, papillary necrosis and calcification. In addition, pelvis dilation and undefined nephropathy were noted in 2-generation studies on sulcotrione.

The RAC does not consider the α2μ-globulin MoA sufficiently well proven to disregard the kidney findings. The observations of pelvis dilation in females as well indicate that α2μ-globulin is not the only MoA, if at all involved. The comparison with sulcotrione provides further support for a class-effect of these HPPD-inhibiting herbicides on the kidney. Kidney effects such as chronic nephropathy, kidney weight increase and dilated renal pelvis are reported as from doses of 0.8 mg/kg/day. The guidance values for STOT RE 2 based on a 2 years study are between 1.25 and 12.5 mg/kg/day, and the kidney effects are sufficiently severe at doses below 12.5 mg/kg/day (see in BD under 'In depth analysis of RAC') to qualify for a STOT RE classification. As to the category, an effect level of 0.8 mg/kg/day is obviously a borderline case between RE 1 and RE 2, but considering that rats are likely to be more sensitive than humans, RAC considers classification in STOT RE 2 appropriate.

Data relevant to the STOT RE classification was also obtained from the developmental study which was performed in rabbits'. Out of 25 dams administered 100 mg/kg/day in a developmental toxicity study, 5 dams died prematurely between gestation day 15 and 22. The effect is unexpected considering the short exposure time (10-17 days). However, mortality was also observed in males in the 90 days dog study at 250 mg/kg/day and in male rats in the 2 year study at 134 mg/kg/day. As no other studies are available in rabbits, it has to be assumed that the rabbit mortality can be explained by a very high sensitivity. Considering the limited effects on the rabbit pups (delayed ossification), there is no reason to believe that the mortality is specific for pregnant rabbits, but rather is a general effect of tembotrione on rabbits. For a short study (28 days), the guidance value is 30-300 mg/kg/day for STOT RE 2. The rabbit mortality is clearly severe and occurs at doses below the relevant guidance value, thus warranting classification with STOT RE 2 (H373).

RAC agreed with the DS proposal that tembotrione should be classified as STOT RE 2 based on mortality seen in rabbits. In addition, RAC concludes that eye, kidney and liver toxicity in rats also warrant classification as STOT RE 2, with the hazard statement; May cause damage to the eye, kidneys and liver trough prolonged or repeated exposure. As there are no repeated dose toxicity studies in any species by the dermal or inhalation route, we cannot exclude the possibility that the substance can exert toxicity by these routes (at least in sensitive rabbits). The RAC therefore considered that the route should not be given in the hazard statement. The corresponding classification according to DSD would be Xn; R48/22.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

The DS did not propose classification for reproductive toxicity. There were no indications of effects on fertility in a 2-generation study in rats. In the developmental studies in rats and rabbits, there were indications of delayed skeletal ossification. However, this delay was thought to be related to the tyrosinaemia, and in rabbits it also occurred in the presence of maternal mortality. Three cases of dilated cerebral ventricles were found at the top dose in the rabbit study, in the absence of any other effects on the CNS, and the effect could therefore be caused by a general delay in the brain development.

Comments received during public consultation

No classification was proposed for reproductive toxicity. Three comments were received. Two member states proposed classification for developmental toxicity and one industry organisation supported no classification.

RAC assessment and comparison with the classification criteria

Fertility

The only effect that could possibly be linked to fertility was a statistically significantly reduced number of corpora lutea in high dose F1 animals of the rat 2-generation study (26.8 vs. 40.3 in controls). There were no effects on ovarian weight, or on number of primordial and antral follicles. No historical control data were given in the CLH report. The CLH report argues that the sole reduction of corpora lutea is not considered an adverse effect. The RAC is of the opinion that a reduced number of corpora lutea can be an adverse finding, but that this isolated finding, in successfully reproducing animals, is not sufficient for classification for effects on fertility.

Developmental toxicity

The pup weights in the rat 2-generation study were not affected at birth, but growth was dose-dependently decreased during the lactation phase by up to 19% (at a dose of 100-200 mg/kg/day), from day 4 and 7 in F1 and F2, respectively. The decreased growth rate was accompanied by developmental delays (time of preputial separation and vaginal opening). Except for ocular toxicity, no other effects were noted in the parental animals. The effects in pups could thus qualify as developmental toxicity or possibly lactational toxicity. Severe ocular effects could be seen in pups of all treated groups, indicating that the pups suffered from tyrosinaemia. Whether the reduced growth rate could be related to the tyrosinaemia is not clear, but cannot be ruled out if a higher sensitivity of young versus older animals is assumed and considering the ocular toxicity observed in the pups.

In the rat developmental toxicity study, pup body weights were significantly and dose-dependently reduced at sacrifice on gestation day 21 by 3, 8 and 16% in low, mid, and high dose groups, respectively (25, 125, and 500 mg/kg/day). Many dose-dependent variations related to poor ossification were noted, some even in the low dose group (without effects on the maternal body weight). However, statistical significance is not reported and historical control incidences are not included in the CLH report. The CLH report refers to a study by Kennel (2006) (see BD) to disregard the skeletal variations. The Kennel study is said to show increased incidences of delayed ossification in rats treated with tyrosine and an HPPD-inhibitor, but no data is given in the CLH report. The full study report was provided during the opinion development process and was assessed by the RAC (see in depth analysis below). The RAC notes that induced tyrosinaemia and tembotrione treatment cause similar effects on body weight and skeletal ossification, and concludes that the skeletal variations caused by tembotrione are caused by the tyrosinaemia.

Delayed ossifications were also found in the rabbit developmental toxicity study, Although maternal body weights were not affected, 20% dam mortality was noted at the highest dose, indicating that developmental effects noted at the top dose (100 mg/kg/day) could be caused by the excessive maternal toxicity and should therefore not be considered for classification. This includes the three findings of dilated cerebral ventricles (in 2 litters), occurring in the absence of other findings in the CNS. However, statistically significantly increased litter incidences of variations (extra ossification sites between atlas and axis centrum, incomplete ossification of pubis) and anomalies (presence of 27 presacral vertebrae in combination with 13 thoracic rib) were also noted at the mid dose (10 mg/kg/day). The incidences of these findings were roughly twice the highest historical control rates. In a separate study, the dose level of 10 mg/kg/day has been shown to lead to elevated concentrations of tyrosine (6-fold) in pregnant rabbits. The CLH report suggests that the delayed ossification is caused by tyrosinaemia, but the effects of induced tyrosinaemia have not been studied in rabbits.

The consistent findings of skeletal variations and anomalies, and of reduced growth of rats during the gestational and lactational phase, with secondary effects on sexual development, do not provide an undisputable argumentation for classification of developmental toxicity. However, although it has not been shown in the CLH report that tyrosinaemia decreases the growth rate, the Kennel study (2006) (see BD) shows that provoked tyrosinaemia decreases the pup body weight and delays the ossification of rats at the time of birth, making it likely that the decreased growth rate also after birth could be related to the tyrosinaemia. This assumption is supported by the occurrence of eye damage in the pups, a key effect of tyrosinaemia, clearly indicating that the pups suffered from tyrosinaemia also during the lactation phase. The corneal opacities were first

observed at day 23, with a similar LOAEL (the lowest dose tested) as in the dams of the 2-generation study.

The RAC therefore concludes that tembotrione affects skeletal development in rats (variations) and rabbits (anomalies and variations), and decreases pre- and postnatal growth rates in rats, at doses not affecting e.g. maternal body weights. The MoA is likely to be tyrosinaemia, leading to effects characterised by a decreased growth rate of the pups.
The criteria state:"*The classification of a substance in category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other effects or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effect. However, when there is mechanistic information which raises doubt about the relevance of the effect for humans, classification in category 2 may be more appropriate."*

The effects are adverse, and could be considered for category 1B, but they are not very severe (decreased growth rate). The adverse effects on reproduction occur at doses causing tyrosinaemia in the dams, and the maternal tyrosinaemia is the likely specific mode of action for the reproductive effects. Thus, the reproductive effects are not considered to be secondary non-specific consequences of other toxic effects. Similarly, the MoA is relevant for humans, but it can also be expected that humans are less sensitive than rats. See also the 'In depth analyses by RAC' of repeated dose toxicity in the BD. Because of these uncertainties, category 1B does not seem appropriate. Still, classification is warranted, and the RAC therefore proposed classification with Repr. 2 - H361d (CLP) (Rep. Cat. 3; R63 according to DSD) in consideration of these uncertainties.

RAC evaluation of environmental hazards

Summary of Dossier submitter's proposal

The DS proposed environmental hazard classification for tembotrione as Aquatic Acute 1, H400 (M=10) and Aquatic Chronic 1, H410 (M=10) according to CLP, and N, R50/53 according to DSD with SCL R50-53 ≥2,5%; R51-53 ≥0,25-<2,5; R52-53 ≥0,025-<0,25.

Degradation
Degradation was studied in a hydrolysis test, a photolysis test, a ready biodegradability test, an aerobic (water/sediment) study, three aerobic (soil) degradation laboratory studies and two anaerobic (soil) degradation laboratory studies.

The DS considered tembotrione as hydrolytically stable and moderately photodegradable with a measured half-life of 56.3 days. It degraded rapidly in air by reaction with OH radicals, although the presence of this compound in air is not expected due to its low vapour pressure.

Tembotrione is not readily biodegradable under test conditions (OECD 301D).

In a water/sediment study tembotrione showed a very slow degradation with a $DT50_{whole\ system}$ of 108 days and in aerobic soil degradation studies tembotrione degraded with a half-life from 4.3 to 56.4 days while in anaerobic conditions the DT_{50} was 278 days.

Based on the available data the DS considered tembotrione as not rapidly degradable.

Bioaccumulation
The log P_{ow} of tembotrione was reported to be 2.2, at pH 2, -1.1 at pH7 and -1.4 at pH 9. Experimental bioconcentration tests are not available. Since the log Pow indicated low potential for bioaccumulation, the DS concluded that tembotrione has low potential for bioaccumulation.

Aquatic toxicity
Three acute toxicity studies in fish, one in invertebrates, three in algae, five in algae and aquatic plants, including *Lemna gibba*, and finally two more tests in marine invertebrates were reported by the DS. One long-term toxicity study in fish (34 days, *Pimephales promelas*), one in aquatic

invertebrates, six in algae and aquatic plants and one more in sediment dwelling organisms (*Chironomus riparius*) were available in the CLH report.

The marine invertebrate (*Americamysis bahia*) was the most sensitive taxonomic group in acute tests, with EC_{50} value of 0.1 mg/l while in chronic tests the most sensitive species was *Lemna gibba,* with a NOErC value of 0.0024 mg/l. These two values were used as key studies for classification.

Comments received during public consultation

Six comments were received regarding the use of the marine invertebrate (*Americamysis bahia*) as the most sensitive taxonomic group in acute tests, with a ErC_{50} value of 0.1 mg/l, when in fact the *Lemna gibba* was the most sensitive specie for acute toxicity with an 7d ErC_{50} of = 0.00848 mg/l.

One commenter questioned the use of a *Lemna gibba* study based on OECD TG 221 performed with sediment, for aquatic chronic classification.

In their post public consultation response the DS agreed that the most sensitive species for acute classification is *Lemna gibba*, and therefore they supported classification for Aquatic Acute 1, H400 (M=100) and Aquatic Chronic 1, H410 (M=10) according to CLP, and N, R50-53 according to DSD with SCL R50-53 ≥0.25%; R51-53 ≥0.025-<0.25; R52-53 ≥0.0025-<0.025.

Regarding the use of studies performed with sediment for classification purposes, the DS stated that it should be discussed by the ECHA experts.

RAC assessment and comparison with criteria

Degradation

RAC agreed that tembotrione can be considered hydrolytically stable and moderately photodegradable based on the information provided in the CLH report.

RAC also agreed that tembotrione is not readily biodegradable under the reported test conditions (OECD 301D). Furthermore, in an aerobic water/sediment study tembotrione shows a very slow degradation ($DT_{50whole\ system}$ =108 days at 20ºC), therefore, based on these data, RAC agrees with the DS that tembotrione must be considered not readily biodegradable according to DSD and not rapidly degradable according to CLP.

Bioaccumulation

In the current CLP criteria (2nd ATP) bioaccumulation is relevant only if the surrogate approach is applied for assessing long-term hazards. For tembotrione, adequate chronic toxicity data is available for all trophic levels and therefore, bioaccumulation data is not used for classification according to CLP. However, under the DSD bioaccumulation should be used for assessing long-term adverse effects. In this case it does not meet the criteria for classification, since the measured log Kow = -1.09 at pH= 7 and 24ºC and therefore lower than 3.

Aquatic toxicity

Under CLP, classification for acute toxicity should be based on the most sensitive species. In the case of tembotrione, that is *Lemna gibba* (Sowig, 2003, see BD), with an ErC50 of 0.00848 mg/l. As the LC_{50} value is below 1 mg/l, the classification should be Acute category 1 – H400. As the value is between 0.001 and 0.01 mg/l, an M factor of 100 is appropriate.

According to section 4.1.3.2.3 of the Guidance on the Application of the CLP Criteria (p.409) *Lemna gibba* studies shall be considered if the test methodology is suitable. The aquatic plant growth inhibition tests are normally considered as chronic tests but the EC_{50}s are treated as acute values for classification purposes.

Regarding chronic toxicity, the lowest NOErC value is reported in a study on *Lemna gibba* based on OECD TG 221 (NOErC= 0.0024mg/l; the value based on mean measured concentration; Dorgerloh, 2004a, see BD). However the test was modified, including sediment in the test system which can modify the recoveries in water. Therefore the NOErC of 0.0032 mg/l

(nominal-recoveries higher than 80%) from the study on *Lemna gibba* (Sowig, 2003, see BD) performed without sediment should be used. Nevertheless, both tests gave roughly the same NOEC value based on nominal concentrations (NOErC: 0.0032 mg/l).
Taking into account the NOEC value of 0.0032 mg/l and its persistence, tembotrione should be classified in Aquatic Chronic category 1 (H410) with an M-factor of 10, because the NOEC value is between 0.001 and 0.01 mg/l.

Under DSD, the key study for acute toxicity has an EC_{50} value of 0.0084 mg/l (*Lemna gibba*), which is below the classification criterion of 1 mg/l and therefore tembotrine should be classified as N; R50.Tembotrine is considered not rapidly degradable and it does not fulfill the criteria of ready degradability (point 5.2.1.3 of Annex 6 of 2001/59/EC). Therefore, classification for long-term adverse effects (R53) under DSD is justified.

RAC agreed with the DS's proposal to classify tembotrione as hazardous to the aquatic environment according to the CLP criteria, however, RAC proposed higher Acute M-factor than in the original proposal by the DS. Classification as Aquatic Acute 1 (H400) with M-Factor 100 and Aquatic Chronic 1 (H410) with M-Factor 10 for tembotrione is warranted (N; R50-53 Specific concentration limits N; R50-53: C ≥ 0,25 %, N; R51-53: 0,025 % ≤ C< 0,25 % and R52-53: 0,0025 % ≤ C< 0,025 % since 0.001 < L(E)C50 ≤ 0.01).

ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. It is based on the CLH report prepared by the dossier submitter; the evaluation performed by the RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report, response to comments provided by the dossier submitter and the RAC (excl. confidential information).